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"HETEROTYPICAL" MITOSIS IN NEREIS LIMBATA (EHLERS).

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As the result of investigations on the chromosomes in *Enteroxenos östergreni* I published some years ago (Bonnevie, 1905, 1906) the following conclusions (p. 384, 1906):

"Die Zahlenreduktion der Chromosomen geschieht bei Enteroxenos durch ihre parallele Konjugation in Synapsis. Die dadurch entstandene Doppelheit der Chromosomen geht weder in der ersten noch in der zweiten Reifungsteilung wieder verloren, sondern tritt noch in den Vorkernen deutlich hervor und verschwindet erst im Laufe der folgenden Zellgenerationen mit der völligen Verschmelzung der konjugierten Chromosomen.

"Die konjugierten Chromosomen haben ihre Teilungsfähigkeit behalten; beide Reifungsteilungen sind somit als Aequationsteilungen zu betrachten, deren Bild jedoch durch die Doppelheit und die Grösze der Chromosomen kompliziert wird. Das rasche Aufeinanderfolgen beider Teilungen trägt zu einer Grószenreduktion der Doppelchromosomen bei; sie werden jedoch erst im Laufe vieler Zellgenerationen auf ihre ursprüngliche Grösze reduziert."

At the time when I first formed this opinion, no clear evidence existed of the corresponding process in other organisms sufficient to make my conclusions seem improbable. But during the time which passed before the publication of my final work a series of papers (Schreiner, 1904, '05; Grégoire, 1904, '05; Montgomery, 1905) appeared, which — though from different points of view — all agreed in claiming for the first maturation mitosis the general significance of a reductional division, separating the chromosomes, which had conjugated at an earlier period.

Opposed to the apparently strong evidence in favor of a reduction division brought together in these papers, as well as that from the valuable investigations of Schreiner (1906*a*) on the maturation process in *Tomopteris* — my results on *Enteroxenos* stood quite isolated. And the reasons which at an earlier period had seemed strong enough to support my view, might now seem inadequate.[1] Even before the appearance of my final paper (1906) I therefore felt the necessity first of reinvestigating the maturation process in *Enteroxenos* and second, in case of the confirmation of my earlier results of finding another object, in which the behavior of the chromosomes might be more easily followed than in this species.

On reinvestigating my old *Enteroxenos*-preparations as well as new material of the same species, I found, that although it might well be possible, even in this species, to select a series of maturation stages showing the "*Tomopteris*-type" (Schreiner, 1906*a*), yet other structures are present in the chromosomes which do not support the assumption of a reduction division.

Besides the great similarity in the general appearance of the two maturation divisions there is a longitudinal split in the chromosomes at the end of this period — a structure which suggests an interrogation as to the assumption of a reduction division, until the existence of this mode of mitosis has been proved for this very species.

On the other hand, however, I willingly admit that *Enteroxenos* is not a favorable object for a decision of these difficult questions — the chromosomes being very much contracted during the metaphase and so small, that the structures in question are often beyond the limit of an objective demonstration. It therefore seemed desirable to extend my investigations to other species with more favorable chromosome relations.

[1] (Added on the proof-sheet, June, 1907.) The truth of this sentence was clearly proved through the appearance of the latest paper by A. and K. E. Schreiner (1907) some weeks ago. Their results seem to prove that "the new observations in my paper were not good, while the good ones (if present) were not new." I hope however, through this and my following publications to show that my main results on the maturation divisions in *Enteroxenos* were correct, and that the doubt which they made me feel with regard to the existence of a reduction division in this species was well founded, even if future observations should show that my interpretation of the new facts would have to be modified.

At Columbia University, New York, where I have spent last winter, I have had the best opportunity of doing this; and I want here to express my most sincere thanks to Professor E. B. Wilson for offering me a table in his laboratory, for his generous liberality in giving me free use of his valuable material, and for the lively interest with which he has followed my work.

In this paper I wish to give a preliminary account of my results on *Nereis limbata* Ehlers, a species in which the chromosomes are especially favorable for an investigation of the mitotic process — results which have obliged me to maintain a position different from that represented in the papers of Grégoire and Schreiner.

The most important of these papers, it seems to me, is that of A. and K. E. Schreiner on *Tomopteris* (1906*a*). In this species they have found an object, in which the whole maturation process of the chromosomes can be followed and demonstrated with an apparently indisputable clearness. After a comparison of their results on *Tomopteris* with the maturation process in other animals and plants (1906*a* and *b*), they find it very probable that (p. 474, 1906*b*) "dieser Process bei allen höheren organischen Wesen von einem gemeinsamen Gesetze geleitet wird, das ihm unter ähnlichen Verhältniszen ein ähnliches Gepräge aufdrückt, und zwar das Gepräge des '*Tomopteris*-Typus'"; and also that (p. 475) "die Zeit nicht fern ist, wo das 'Reduktionsproblem' von morphologischem Gesichtspunkte aus als gelöst angesehen werden darf."

I fully agree with A. and K. E. Schreiner, that the knowledge of the maturation process in *Tomopteris* is of great importance for our understanding of the same period in other organisms. Especially valuable seems to me their convincing demonstration of a parallel conjugation of the chromosomes in this species and their identification of the same process in so many other groups.[1] Of great value also are the demonstrations of Grégoire

[1] I have reason to believe that the conjugation process in *Enteroxenos* follows the type of *Tomopteris* more closely, than is shown in my figures (Bonnevie, 1906, fig. 33–42 and 159–162). During my first investigation of the spermatocytes of this species I both observed and figured stages, in which there was a parallel arrangement of thin chromatic threads, the ends of which were directed towards one pole of the nucleus; and it was, in fact, these pictures which made me join von Winiwarter

(1905) and of Schreiner (1906*a* and *b*) of the close resemblance between the chromosomes of very different species during the first maturation mitosis; and certainly, the presence of this same type throughout the whole animal (and plant) kingdoms cannot but give one the impression that (Schreiner, *loc. cit.*) "dieser Process bei allen höheren organischen Wesen von einem gemeinsamen Gesetze geleitet wird."

But what is the law that determines the behavior of the chromosomes during the maturation period?

In my opinion the answer to this question is not yet given, in spite of the apparently overwhelming evidence brought together in the works of Grégoire and Schreiner to demonstrate that the first maturation division is always a heterotypical one, and that this heterotypical character finds its explanation in the fact, that (Schreiner, 1906*a*, p. 44) "hier Ganzchromosomen, die nie mit einander eine Einheitlichkeit gebildet haben, von einander getrennt werden."

My results in *Nereis* will, however, clearly demonstrate that a "heterotypical" mitosis cannot be considered as identical with a reductional one; and I hope to show in the following pages that the problem of the reduction of the chromosomes is still entirely open to discussion.

My material consisted of a most valuable series of maturation and segmentation stages of *Nereis limbata*, collected by Professor E. B. Wilson at Woods Hole, in 1896–97. The sections as well as the uncut material, fixed partly in picro-acetic acid, partly in Flemming's fluid, proved to be still in perfectly good condition; and the material contains an uninterrupted series of stages, from the moment of fertilization to the four-cell stage, and also the later segmentation stages, up to fifteen and one half hours after fertilization, these, however, with intervals in their development of two to five hours.

(1901) in his hypothesis of a parallel conjugation. As I, however, found very few cells of the same appearance among the young oöcytes, I did not believe this to be the typical arrangement of the chromatin; and I therefore tried to find other stages showing the first traces of a parallelism between the thin threads. In the light of the chromosome relations in *Tomopteris* I now think it probable, that a reinvestigation of new material of *Enteroxenos* will give results somewhat different from those shown in my paper. (Added June, 1907.) This supposition is confirmed by Schreiner, 1907.

The maturation process in the egg of *Nereis* does not begin until fertilization has taken place, and the earliest stages contained in my material show the nuclear membrane still unbroken, while outside of it two small asters have made their first appearance. Within the large nucleus fourteen chromosomes are found scattered around, most of them, however, lying relatively near to the nuclear membrane.

The chromosomes appear in shapes, well known from other worms — *Allolobophora* (Foot and Strobell, 1905), *Tomopteris* (Schreiner, 1906*a*) and others — forming rings and crosses of different kinds; but they also very often appear in a more irregular shape. (See earl. proph. of 1st mat. div.; p. 62.)

A comparison of these different chromosome forms shows

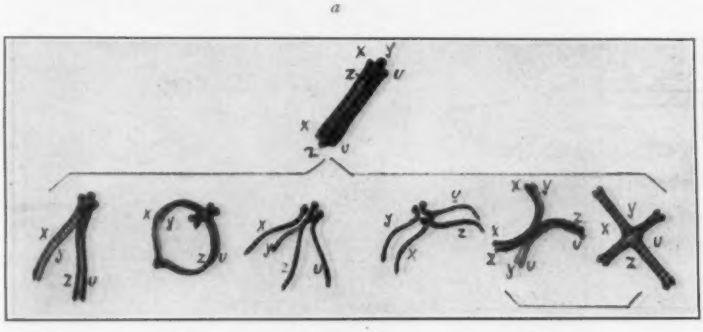
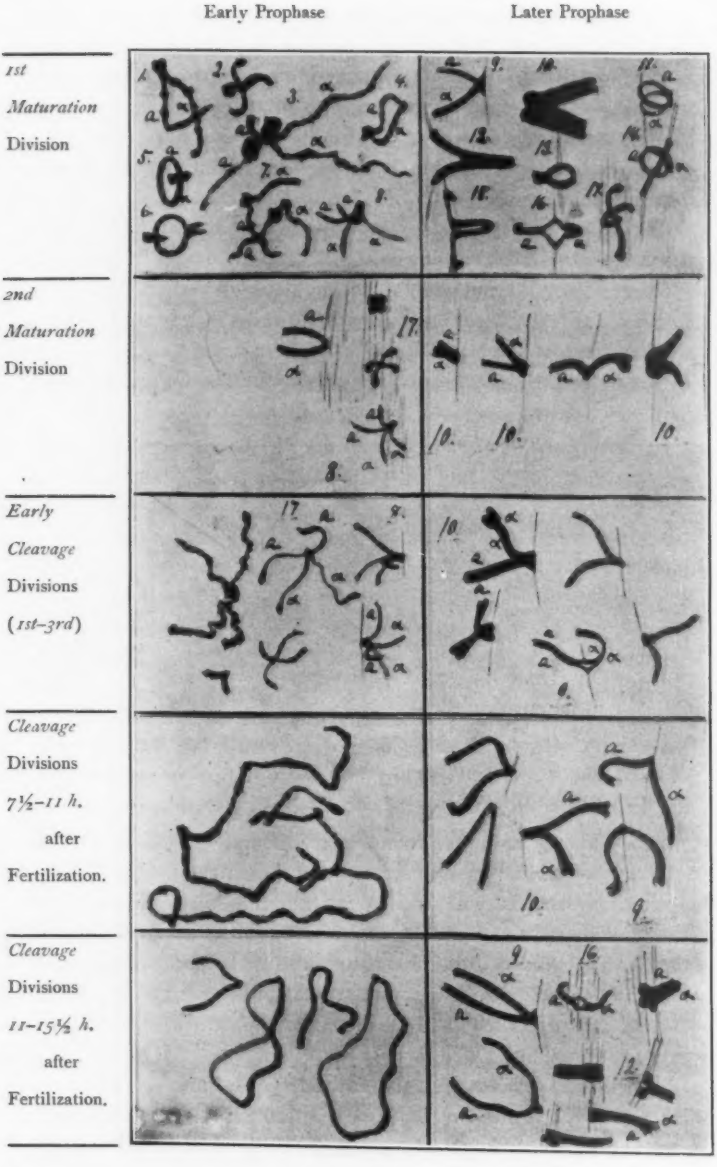


FIG. 1, *a–g*. Schematic illustration of the development of chromosomes from the original tetrad. Explanation in the text.

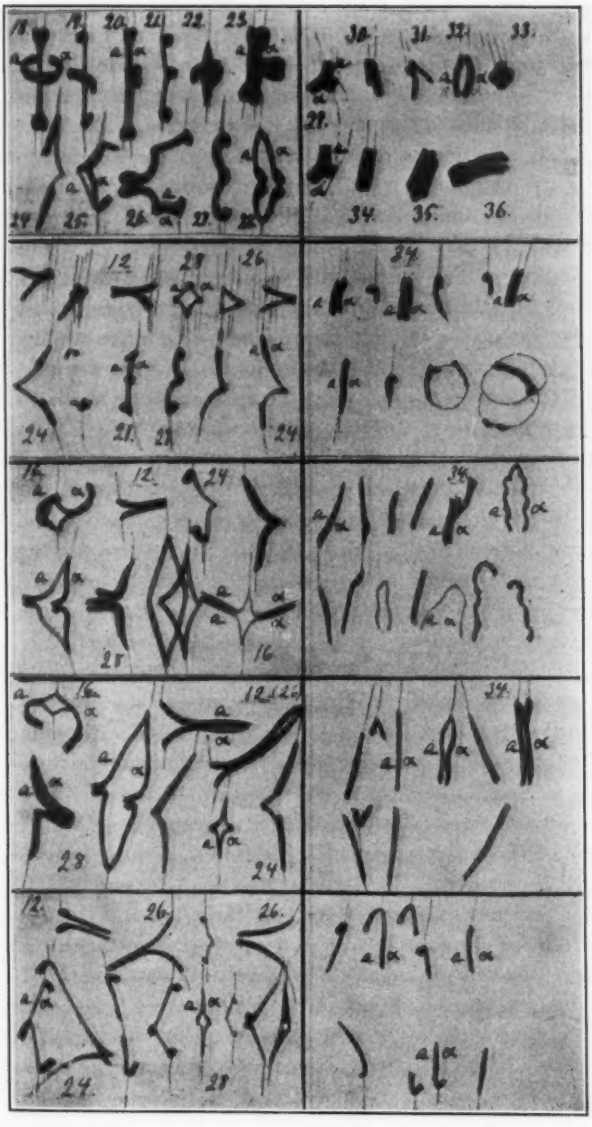
that they all are reducible to one and the same type — to a more or less elongated tetrad (Fig. 1, *a*) in which the four originally parallel elements may be arranged in different ways (Fig. 1, *b–g*).

In most cases the four elements are combined in pairs, so as to give the appearance of two longitudinally split ribbons, connected at one (Fig. 1, *b*; chrom. 1, p. 62), or at both ends; in the latter case the chromosomes form more or less typical rings (Fig. 1, *c*; chrom. 4–6, p. 62).

In other tetrads we find the four elements connected at one end, but diverging from this point in different directions (Fig. 1, *d*). Such an arrangement gives rise to cross-shaped chromo-


Early Prophase
Later Prophase
1st Maturation Division
2nd Maturation Division
Early Cleavage Divisions (1st–3rd)
Cleavage Divisions 7½–11 h. after Fertilization.
Cleavage Divisions 11–15½ h. after Fertilization.

Early Anaphase Later Anaphase



1st *Maturation* Division

2nd *Maturation* Division

Early *Cleavage* Divisions (*1st–3rd*)

Cleavage Divisions *7½–11 h.* after Fertilization.

Cleavage Divisions *11–15½ h.* after Fertilization.

somes with four single arms, all of the same length and usually extending to one and the same side of their connecting point (chrom. 3, 8, p. 62).

Another kind of cross — double-armed ones — may also be derived from the original tetrad, its four elements being arranged in pairs, but combined in a different way at each end of the tetrad (Fig. 1, *f*. At the upper end the elements $x + y$ are diverging from $z + u$, at the other end $x + z$ remain parallel, diverging from $y + u$.) Through a flattening down of a figure, formed in this way we get a cross-shaped chromosome (Fig. 1, *g*), whose arms appear longitudinally split, and in which always two arms, lying opposite to each other, are of the same length. Sometimes this may be the case with all four arms, but more often we find a considerable difference between the two pairs. In many chromosomes this difference is so great that the short arms of the cross appear only like a pair of lateral projections on the middle of a longitudinally split ribbon; and from those forms there is a very short step to the chromosomes represented in Fig. 1, *b* and *c*.

Finally we may often find chromosomes consisting of two apparently separate halves (Fig. 1, *e*; chrom. 7, p. 62). Here also the origin of the chromosome may be traced back to a tetrad, and most easily through a transition form like that in Fig. 1, *b*, the arms of such a chromosome being divided along their longitudinal split.

A comparison of the whole chromosome group in a number of nuclei shows that these different forms are not characteristic of special chromosomes. I have found nuclei, in which all the chromosomes were cross-shaped, others in which two or three rings were present among the crosses, and again others, in which one or both of these forms were mingled with the more irregularly formed chromosomes.

Nor did I find any evidence in favor of the view, that the different forms of the chromosomes should represent different stages in their development. It seems more probable that the rings, the two kinds of crosses, the rodlike chromosomes, etc., arise simultaneously from the original tetrads and that their special shape is more a result of chance — possibly of their conditions within the nucleus — than of any individual character of the

chromosome. The formation of rings seems, however, to be limited to chromosomes of a certain size.

At the time of the dissolution of the nuclear membrane the chromosomes are found to be slightly contracted, and while some of them regain their original tetrad-form, others remain like V- or horseshoe-shaped rods, longitudinally split and with a thickening at their middle point. Also the ring-shaped chromosomes usually retain their form, while the numerous crosses, found within the nucleus, are transformed into tetrads or V-shaped chromosomes. In only one case were two cross-shaped chromosomes found attached to an early spindle.

Considering the chromosomes of the prophase as different modifications of the tetrad, we will find that their attachment to the spindle-fibers is in all cases a terminal or a slightly subterminal one. (See lat. proph. of 1st mat. div., p. 62).

The unmodified tetrads are attached at one end, their longitudinally split halves being separated from each other (chrom. 12, 15).

The V- and horseshoe-shaped chromosomes are attached at their middle point, this representing one end of the original tetrad (chrom. 9, 10, 16).

The rings are placed horizontally [1] on the spindle and the fibers attached either at their transverse projections (chrom. 14) or at the point opposite to these (chrom. 11).

In each case the attachment is a terminal one, and the rings, as also the V-shaped chromosomes, are divided along a plane represented by their longitudinal split. In the above mentioned case in which I have seen cross-shaped chromosomes attached to the early spindle, the point of attachment seemed to be at their center, all four arms being bent in a direction away from the axis of the spindle (chrom. 17, p. 62). Considering the crosses as tetrads with four diverging elements, we find here also a terminal attachment of the fibers.

Besides the forms already mentioned I also found, in two or three cases, ring-shaped chromosomes placed in such a way on the spindle that they must be divided into two half-rings (chrom. 13, p. 62). In all of these cases, however, the rings were smaller

[1] In the following description the axis of the spindle is always supposed to have a vertical position.

than those found within the nucleus, and which we have seen placed horizontally on the spindle. I consider, therefore, that this is undoubtedly a secondary ring formation, caused by a subterminal attachment of the fibers to a tetrad-shaped chromosome.[1]

The early prophase, in which the chromosomes are yet quite irregularly scattered on the surface of the spindle, is, according to the above stated facts, characterized by an arrangement of the chromosomes at right angles to the axis of the spindle.

This stage is followed by another, in which this horizontal position of the chromosomes is gradually changed into a vertical one, the daughter chromosomes being pulled towards each pole of the spindle (see earl. anaph. of 1st mat. div., p. 63).

During the time in which this separation takes place the chromosomes very often pass through a second cross-like stage (chrom. 18, 19, 22), the fibers being attached at or near the ends of the vertical arms of the cross. At first the horizontal arms are relatively long; a longitudinal split may be clearly visible in them, and they are often bent (in a direction away from

[1] The behavior of the ring-shaped chromosomes in *Nereis* confirms my conclusion from *Enteroxenos* (Bonnevie, 1905, 1906) that the rings of the prophase cannot always be considered as identical with those of the metaphase. The prophase rings are in *Nereis* divided in their own plane, and during the separation of the daughter-chromosomes other rings are transiently formed from tetrads and V-shaped chromosomes; these metaphase rings are then sooner or later divided into two half rings.

According to a note in their latest work A. and K. E. Schreiner (1906*b*, p. 442) seem to have observed metaphase-rings in the first maturation division of *Enteroxenos*. This fact does, however, neither change nor contradict my results on the same species, that other rings are divided in their own plane and thus still appear as rings in the telophase.

In the same paper (Schreiner, 1906*b*, p. 444), is found the following phrase:

"Die Verfasser (Farmer u. Moore) meinen jetzt, wie Montgomery und Bonnevie, dass die bivalenten Chromosomen nicht durch Spaltung der in reduzierter Zahl vorhandenen Schlingen, sondern durch Zussammenbiegung derselben gebildet werden."

Because of this misleading account I want here once more to state my exact position with regard to these questions. I have described the bivalent chromosomes arising through a parallel conjugation of two homologous univalent ones — a view which is very different from that held by Montgomery and by Farmer and Moore. And with regard to ring-shaped chromosomes, I have shown that they may be formed in different ways, through an approach of the free ends of a chromosome (postsynapsis of *Enteroxenos*), or through a widening of a longitudinal split, while the two halves of the chromosome are still connected at their ends (cleavage division of *Enteroxenos* and many cases described in the literature). And further, that from the presence of ring-shaped chromosomes no conclusions can be drawn with regard to the nature of the mitosis, as it has been shown that rings may divide in two different planes.

the axis of the spindle) so as to form a more or less nearly closed ring (chrom. 18, 22).

The vertical arms of the cross on the other hand, are at first short, and their elongation evidently takes place at the cost of the horizontal arms. They are in most cases thinner than the horizontal arms (except their endpiece, if this is lying beyond the point of attachment of the fibers), and very often no longitudinal split can be seen in this part of the chromosomes (chrom. 19, 21, 24, 27). It is clear, however, from their earlier as well as from their later stages, that a doubleness is present even here; and when it is not visible, it may be due to the stretching of the vertical arms of the chromosomes.

Metaphase rings seem to be formed mostly from horseshoe-shaped chromosomes (chrom. 9, 16, 28). They are divided into two half rings. I have never observed a longitudinal split in these half rings; and an examination of a great number of chromosomes shows that the metaphase-rings of *Nereis* are to be directly compared with the cross-shaped chromosomes of the same stage, the space between the two branches of each half ring being identical with the longitudinal split in the vertical arms of the cross. Very often we find this space in the rings filled by an achromatic substance (like the "Zwischensubstanz" of the chromosomes of *Enteroxenos*);[1] and we find, indeed, all transition stages between the open rings and the thin vertically stretched arms of the crosses.

[1] (Added on the proof-sheet, June, 1907.) The existence of such a substance in the chromosomes of *Enteroxenos* is absolutely denied by A. and K. E. Schreiner (1907). They say (p. 12):

"Wir haben an unseren Präparaten vergebens nach dieser sehr eigenthümlichen Substanz gesucht, und es scheint uns unzweifelhaft, dass sich Bonnevie in diesem Punkt vollkommen getäuscht hat, indem sie bald die Spalte zwischen den Komponenten eines Doppelchromosoms, bald die Längslichtung in den Komponenten selbst, bald aber auch achromatische Verbindungen zwischen zwei oder mehreren Chromosomen als eine Kittmasse gedeutet hat."

In the face of this sweeping criticism I can only repeat that in *Enteroxenos*, as well as in *Nereis*, *Thalassema* and *Doris*, I have found the chromosomes of the maturation divisions, and especially those of the first, containing an achromatic substance which can be stretched out to a considerable width between the branches of the chromosomes.

In this respect the chromosomes of the maturation differ from those of other divisions in the same material.

If this substance has not been visible in Schreiner's preparations, it must depend upon its being dissolved or contracted.

After their separation the daughter chromosomes contract to relatively short and thick rods, in which the longitudinal split is in most cases clearly visible (see p. 63, lat. anaph. of 1st mat. div.). Sometimes the two halves of these chromosomes show a tendency to diverge from each other at their free end (chrom. 31); and once I have found a pair of daughter chromosomes (chrom. 29) in which a double longitudinal split seemed present — the one which is usually visible (separating *a* from *a*) and on the inside of the diverging halves of the chromosomes another split at right angles to the first one.

Such a tetrad-like appearance of the daughter chromosomes is found more often in the telophase (chrom. 35, 36). But I have not been able to decide with certainty, whether or not this appearance is due to a mere surface structure. An examination of the following stages, however, makes it very probable that these chromosomes ought to be considered as real tetrads.

Reviewing the different stages of the first maturation division, we find:

That the point of attachment of the daughter chromosomes corresponds to a point at (or near) the end of the original tetrad.

That the plane of division was represented by the longitudinal split of the rings and the V-shaped chromosomes of the early prophase — and

That the longitudinal split of the daughter chromosomes is identical with the space between the two arms of the V-shaped chromosomes.

What has been said about the chromosomes of the first maturation division may with some modifications be applied also to any of the following divisions up to fifteen and one half hours after fertilization.[1]

The elongated chromosomes are before each division placed horizontally on the (vertical) spindle, and — transiently appearing like rings, crosses, E-shaped chromosomes, etc., — they are before the separation of the daughter chromosomes carried into a position parallel to the spindle fibers.

[1] Whether the same type of mitosis may be found throughout the whole life of the animal, I am not yet able to say.

Up to eleven hours after the fertilization a longitudinal split may be clearly visible in the terminally (or subterminally) attached daughter chromosomes, this split being as in the first maturation division identical with the space between the two branches of a metaphase-halfring or, which is the same, between the two diverging arms of the horseshoe-shaped chromosomes of the prophase.

With regard to this period, therefore, it will be enough to mention the characteristics through which each division, or group of divisions, is distinguished from the other ones (comp. the chrom. of the different divisions on pp. 62–63).

Second Maturation Division.

In the eggs of *Nereis* there is no resting stage between the two maturation divisions, the formation of a new spindle taking place even before the first polocyte is fully separated from the egg-cell. The chromosomes, therefore, pass directly from the telophase of the first division into the prophase of the second, and no great changes are seen to take place in their structure (see p. 62, 2nd mat. div.).

The chromosomes of the prophase are mostly V- or horseshoe-shaped with a longitudinal split and attached by their middle point. There are however also found cross-shaped ones with four equally long arms being attached by their center on the surface of the spindle. In the metaphase we find the chromosomes arranged in a circle round the equator of the spindle, their form being through an approach of the diverging arms, transformed into a rodlike one. In a few cases I have seen a tetrad-like structure of these chromosomes (chrom. 12, p. 63) a fact which is in complete harmony with their genesis and with the appearance of a longitudinal split in the daughter chromosomes.[1]

[1] (Added on the proof-sheet, June, 1907.) With regard to a similar doubleness of the chromosomes shown by me (1905, '06) in *Enteroxenos*, A. and K. E. Schreiner express themselves as follows (1907, p. 18):

"Weder die Beobachtungen Bonnevie's von dem Vorhandensein einer solchen Doppelheit der Chromosomen, noch ihr Versuch, dieselbe mit der in der I. Reifungsteilung sichtbaren zu vergleichen, sind neu; vielmehr gibt es schon über diese Frage eine ganze kleine Literatur, die aber von Bonnevie nur geringe Beobachtungen gefunden hat."

They then mention the observations of Ed. van Beneden (1883) and Flemming

In the telophase the chromosomes grow longer and thinner, their longitudinal split often disappearing; and small drops of hyaloplasm are seen accumulating at the side of each of them, or between two neighboring chromosomes (lat. anaph. of 2d mat. div.). Through the growth and fusion of these vacuoles the female pronucleus[1] is formed; and the chromosomes, invariably adhering to the surface of the vacuoles, soon lose their staining power so that they cannot be distinguished within the resting pronucleus.

Early Cleavage Divisions.

In the early prophase of these divisions the chromatic substance of the nucleus appears in form of an irregular network, which, however, soon proves to consist of a number (28) of cross-shaped chromosomes, each with four equally long arms without any longitudinal split, and many of them with a conspicuous thickening at the center (earl. proph. of earl. cleav. div., p. 62).

These crosses, attached on the young spindle by their middle-point, are in later stages transformed into V-shaped, longitudinally split chromosomes — a transformation, which can only be due to an approach of two arms of the primary crosses on each side of their point of attachment.

The metaphase of these divisions differ from the maturation di-

(1887), both referred to in my final paper (1906, p. 390),—and besides these also those of Hof (1898) and Merriman (1904) on vegetative cell-divisions in plants.

According to observations, to be published in a following paper, I can now say, from my own experience, that this doubleness, occasionally mentioned as occurring outside of the maturation period is (with exception perhaps of Van Beneden's observations in the segmentation divisions of *Ascaris*), something quite different from the doubleness of the chromosomes at the end of the maturation period first found by me in *Enteroxenos* — and now also in *Nereis*, *Thalassema* and *Doris*.

What Hof (1898) has seen in "den eben fertig gebildeten Tochterkernen," is the same structure, which is later described by Merriman (1904). She, however, neither has, nor pretends to have, seen a real doubleness of the daughter chromosomes; and her comparison with the maturation phenomena consists in suggesting that also the doubleness so often described at this stage should be (p. 202) "due to the changing of the daughter-chromosomes from tubular structures into the quadripartite threads."

I must, therefore, insist upon the priority of having shown a doubleness of the chromosomes at the end of the maturation divisions. After the appearance of my preliminary account (1905), however, similar structures were shown to exist in *Myxine* (Schreiner, 1905), in *Ascaris mystax* (Marcus, 1905) and in *Dytiscus* (Schäfer, 1907).

[1] The male pronucleus also develops at the same time and in a similar way.

visions, as well as from the later cleavage divisions, practically all the chromosomes retaining their V- or horseshoe-shape and accordingly also their median point of attachment on the spindle (lat. proph. of earl. cleav. div., p. 62). In the early anaphase we therefore here find a greater number of ring-shaped chromosomes than in any of the other divisions.

The aspect of the later anaphase seems at first to form a striking contrast to that of earlier stages, the daughter chromosomes now being rodlike and terminally attached to the fibers. An explanation is, however, found in the fact, that all these chromosomes show a more or less clearly visible longitudinal split, which — as shown by the genesis of the chromosomes — is identical with the space between the two branches of a half ring.

In several cases this split extends all through the daughter chromosomes, so that they lose their V-shape, the two halves being quite separate from each other (chrom. 34, p. 63).

In the telophase the vacuoles are as a rule formed at the side of such a double chromosome or between two neighboring ones.

Later Cleavage Divisions.

(a) Seven and One Half to Eleven Hours After Fertilization.

In the prophase of the later cleavage divisions we miss the cross-shaped stage of the chromosomes. They appear within the nucleus as longitudinally split ribbons (earl. proph., p. 62); and at the time of attachment to the spindle fibers they are, without exception, V- or horseshoe-shaped, being attached by their middle point.

The appearance of the later stages is, up to about nine hours after fertilization, very much like that of the early cleavage, the chromosomes retaining their prophase shape, until the daughter chromosomes are separated.

After this time, however, the aspect of the metaphase is changed through a tendency in the two arms of the V-shaped chromosomes to approach (chrom. 12 (26), earl. anaph., p. 63).

(*b*) *Eleven to Fifteen and One Half Hours After Fertilization.*

The change just mentioned, proceeds rapidly, and fifteen and one half hours after fertilization the metaphase of the mitosis has an appearance very much like that of the second maturation division. The 28 chromosomes, having in the prophase passed through a V-shaped stage (chrom. 9, 16, later proph., p. 62), are in the metaphase forming as many terminally attached tetrads, standing stiffly out from the spindle (chrom. 12).

Most of these tetrads show a considerable thickening of their four elements at their inner end; and a comparison with later stages of the mitosis makes it probable, that this phenomenon is in some causal connection with a dislocation of the point of attachment to the fibers. (See below, p. 74.)

In accordance with the rodlike shape of the chromosomes no open rings were found in the early anaphase. The doubleness of the daughter chromosomes is, however, still often indicated by narrow openings between their two branches during the separation of the daughter chromosomes (chrom. 28, earl. anaph., p. 63).

In the later anaphase no longitudinal split was visible in the daughter chromosomes.

These are the main facts observed in the material of *Nereis* now in my hand. The series of stages is, however, not yet complete, and I therefore prefer to postpone a general discussion of the bearing of my results, until I have had an opportunity of examining the nature of the mitosis also at the end of the germ track, and of comparing the chromosomes of *Nereis* with those of some other types.

On this occasion I only wish to draw the conclusions reached through the examination of the maturation and cleavage division in *Nereis*, and also to point out some questions, the answer to which will be of importance for an understanding of the chromosome relations in this species.

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1. As already mentioned, the behavior of the chromosomes in all divisions in question is practically the same. Their point

of attachment, their plane of division, their typical changes of form during the separation of the daughter chromosomes and the longitudinal split of these chromosomes — these are characters common to all the divisions, taking place within fifteen hours after fertilization.

And yet, every division (or group of divisions) has its characteristic appearance, each representing one step in a series of transformations following the stage of the conjugation of the chromosomes.

The first maturation division is characterized by a great variability in the shape of the chromosomes and also in the time of the separation of the daughter chromosomes. No stage in this division can be considered as the metaphase of the whole mitotic figure, each chromosome passing through its characteristic stages independently of all the others, and without being placed in a typical equatorial plate. Characteristic also is a peculiarity in the (chemical or physical?) structure of the chromosomes, making them appear less stiff and consistent than in other divisions.[1] Thus the daughter chromosomes are by their separation very often sharply bent, their ends forming right angles with each other (chrom. 18–25, 1st mat. div., p. 63), and there is also a marked tendency towards a spherical shape of the free ends of the chromosomes.

Taking the first maturation division as a starting point, all the changes in the mitotic figures going on within 15–16 hours after fertilization may be looked upon as a gradual return from these irregularities to the normal mitosis.[2]

The chromosomes regain slowly their original more rigid structure; and their cross-shape during the separation of the daughter chromosomes becomes less conspicuous to the same degree as the rigidity increases.

Of great interest are also the gradual changes of the chromo-

[1] Meves (1897) and Kingsbury (1902) have drawn quite similar conclusions from the form of the daughter chromosomes in the first maturation mitosis of amphibians.

[2] The second maturation division does not form a good link in this series of transformations, its whole appearance being more like the later cleavage-divisions than the earlier ones. This is, however, a natural consequence of the lack of a resting stage before this division, the changes of the chromosomes within the nucleus being of importance for their appearance during the mitosis.

somes in the early prophase. The prophase of the first maturation mitosis is in most objects characterized by a more or less complete separation of the longitudinal halves of the chromosomes — and in *Nereis* this separation may take place along both longitudinal splits of the original tetrads. Also with regard to this character we find a gradual return to the general type. In the nuclei of the early blastomeres we still find a divergence of the four arms of the tetrad, although here without the variety in form characteristic of the first maturation division. Through an approach of each two arms of the prophase-crosses, the V-shaped chromosomes of the metaphase arise; and in later cleavage-divisions the V-shaped chromosomes of the early prophase are transformed into rod-like tetrads through a similar approach of their arms.

2. Another result of general interest, reached through a comparison of the maturation and cleavage divisions in *Nereis*, concerns the point of attachment of the spindle fibers to the daughter chromosomes.

Although it seems, that the first connection between chromosomes and fibers always takes place at homologous points of the chromosomes, there is a very strong evidence in favor of the assumption that this point is changed during the mitosis.[1]

In the cleavage divisions all the chromosomes are derived from the cross- or V-shaped chromosomes of the prophase, being attached at their middle point. If, therefore, the point of attachment is a fixed one, a subterminal attachment of the daughter chromosomes would seem absolutely excluded. According to the degree of opening of the longitudinal split the attachment of the daughter chromosomes might be called a median or a terminal one, but any intermediate attachment would seem impossible. And yet, we almost always find some of these chromosomes subterminally attached — not so often in the early cleavage as in the maturation and in the later cleavage divisions, where more than half of the daughter chromosomes are often found to be subterminally attached (see p. 63).

An indication of the way in which this dislocation of the fibers

[1] This probability was, from another point of view first suggested by Schreiner, 1906*b*, p. 433.

takes place is given in the later cleavage divisions. The terminal attachment of the tetrads in the metaphase is identical with the median one of the V-shaped chromosomes in the prophase. The tetrads are, however, as it were, pressed against the fibers of the spindle, their proximal ends being thickened or curved (p. 62). The point of attachment of the fibers is in this way changed from a terminal into a subterminal one, the thickened ends of the tetrads being found again as the short branch of the subterminally attached daughter chromosomes. In some cases it seems as if the point of attachment during the early anaphase is still further removed from the end of the chromosome — a dislocation probably due to the fact, that the median part of the daughter chromosomes makes less resistance against a separation than the ends. (See the triangular chrom.; sec. mat. div., p. 63).

3. What is the bearing of the chromosome-relations in *Nereis* with regard to the assumption of a universally existing reduction division?

In the papers of Schreiner (1904, 1906*a* and *b*), Grégoire (1905) and Montgomery (1905) the universality of a prereductional mode of maturation is based upon the similarity between the chromosomes of different species, and more especially on the general appearance of a "heterotypical" mitosis in the first maturation division.

Grégoire (1905) in his valuable review of the literature concerning the maturation divisions in plants and animals, tries to explain the phenomena in question as following his "hétérohoméotypique" scheme — the first maturation division being a heterotypical, the second a homeotypical one.

His provisional definition of these two modes of mitosis and his opinion with regard to their bearing is found in the following sentences (*loc. cit.*, p. 254).

"Pour la période qui nous occupe, la caractéristique de l'hétérotypie consiste dans la division longitudinale anaphasique; la caracteristique de l'homéotypie réside en ce que les chromosomes-filles de cette cinèse sont preparés dès la cinèse précédente par une division longitudinale."

". . . ce schéma, . . ., s'oppose directement au processus *postréductionnel*, mais il laisse ouverte la question du processus *prérèductionnel* et du processus *eumitotique*."

(P. 362): " Disons-le dès maintenant, c'est le *schéma préréductionnel* dont nous espérons démontrer la réalité."

A. and K. E. Schreiner who in their main results fully agree with Grégoire, characterize the heterotypical appearance of the first maturation division in *Tomopteris* as follows (1906*a*, p. 44): " Geht man . . . auf eine genauere Betrachtung des Verhaltens der Chromosomen in den Reifungsteilungen und auf einen Vergleich desselben mit dem Verhalten der Chromosomen in anderen Teilungen ein, so kann es ja keinem Beobachter entgehen, dass die I Reifungsteilung bei fast allen Objekten, wo es gelungen ist, die Struktur der Chromosomen zu analyzieren, einen gemeinsamen Typus zeigt, der sich von dem Typus aller anderen Teilungen in charakteristischer Weise unterscheidet; und zwar besteht der Unterschied darin, dass sich die Schwesterelemente der einzelnen Chromatinportionen bei dieser Teilung schon lange vor dem Eintreten der Mitose . . . in weiter Ausdehnung von einander trennen und während der ganzen Pro- und Metaphase eine viel gröszere Selbständigkeit zeigen, als in irgend einer anderen Teilung der Fall ist. Auch sind die Verbindungen zwischen den Schwesterelementen in dieser Teilung von ganz anderer Art als bei allen anderen Teilungen." . . . " Es scheint uns, dass das Auftreten dieser eigenthümlichen Bilder der Chromosomen während der ersten Teilung nach dem Eintreten der Zahlenreduktion nur dadurch befriedigend erklärt werden kann, dass hier Ganzchromosomen, die nie miteinander eine Einheitlichkeit gebildet haben, von einander getrennt werden."

These considerations represent, according to the authors, the main reasons for their assumption of a prereductional maturation in *Tomopteris*, as it " allein aus der Betrachtung der verschiedenen Längsteilungen kaum möglich (ist) zu einer endgültigen Lösung dieser Frage zu gelangen."

Their assumption of a universality of this mode of maturation, they base upon the general appearance of the " *Tomopteris*-typus " also in other species. So much weight is laid upon this similarity, that the heterotypical character of a few chromosomes is considered a sufficient proof of a reductional nature of the mitosis. Thus in their description of the maturation divisions of *Myxine*, we find (1906*b*, p. 459):

"Nach der Einstellung in die Teilungsebene zeigen die Chromosomen in gewissen Fällen die für die I Reifungsmitose so charakteristischen, in die Äquatorialebene fallenden Verdikkungen und seitlichen Ausläufer, die sicher beweisen, dass es die Spalthälften der bivalenten Schlingen, die Konjuganten, sind, die hier getrennt werden."

The above cited sentences of Grégoire and Schreiner contain the view which forms the basis in their generalizations with regard to the reduction division.

Judging the chromosome relations in *Nereis* from the same point of view, we should find a reduction of the chromosomes taking place not only in each maturation division but also in each of a whole series of cleavage divisions. The early separation of the daughter chromosomes, the heterotypical shape of the chromosomes in metaphase, and their doubleness in the anaphase, are characters common to all these divisions.

This result is so clear and its consequences are so evident, that a further discussion upon this point would seem unnecessary. Before the question about the nature of the maturation divisions can be considered ripe for new generalizations, it will be necessary to widen the base of our investigations to a comparative study not only of the maturation process itself, but also of the changes of the chromosomes during the time following this period.

Though, however, the main base of the modern generalizations is removed through the knowledge of the chromosome relations in *Nereis*, it is not therefore excluded, that a reduction division may exist in this species as well as in others; and we now finally turn to the question:

4. How is the maturation process in *Nereis* to be understood?

In answering this question three different possibilities must be considered, all of which have already been applied for the maturation process in other species.

(*a*) One of the maturation divisions is a reductional one, separating chromosomes, which have conjugated at an earlier period.

(*b*) Both maturation divisions are to be considered as ordinary mitoses, the conjugating chromosomes having fused completely with each other (Boveri, 1890).

(*c*) The conjugating chromosomes do not separate again; but their fusion may proceed so slowly that the appearance of the following divisions is influenced by the doubleness of the chromosomes (Bonnevie, 1905, '06).

Of these three possibilities only the first one will be treated fully in this paper.

According to the concordant results of modern investigators the two longitudinally split halves of the chromosomes in the prophase of the first maturation mitosis (*a* — *a*, p. 62) are to be considered as two conjugating chromosomes united to form a bivalent one.[1]

If, therefore, these two halves were separated from each other in the first maturation division, then this division must with great probability be considered as a reductional one, and all the similar structures in the following divisions would have to be explained in some other way.

Such a conclusion might, in *Nereis*, as in so many other objects, easily be drawn, if the chromosomes of the early prophase are compared with those of the early anaphase; it would, indeed, seem very natural to consider the thickening in the equator of metaphase (or anaphase) chromosomes as identical with that connecting their two halves in the early prophase. But between these two stages there is another, the stage in which the chromosomes are first attached on the young spindle,[2] showing that the long axes of the chromosomes are placed at right angles to the axis of the spindle, that they are attached to the fibers at the connection point between their two halves and divided in a plane represented by their longitudinal split, and finally, that their position parallel to the spindle fibers is secondary — reached during the separation of their daughter chromosomes.

If, therefore, the assumption is correct, that each half of the chromosomes of the prophase represents one of the conjugating chromosomes, then the same must be true of the daughter chro-

[1] It makes here no difference, whether the conjugation of the chromosomes is considered as a parallel one or as having taken place "end to end"; in each case the connection between the two conjugates is supposed to be of the same kind at a stage directly preceding the maturation divisions.

[2] As will be shown in my final paper, there is no escape from this fact through the suggestion that I should have "confondu les stades" (Gregoire, 1904, p. 307).

mosomes; and *the first maturation division is certainly not a reduction division.*

The possibility, however, of the second maturation division of *Nereis* being a reductional mitosis, is not absolutely excluded, although the only reason for accepting this view must, as far as I can see, be sought in a preconceived assumption in favor of such a mode of maturation.

The chromosomes of the prophase show in the second maturation division, as in the first, and in the early cleavage divisions, two longitudinal splits, one of which is identical with the split of the daughter chromosomes of the first division, the other being a new formation generally not appearing till in the prophase of the second but sometimes indicated as early as in the anaphase of the first division. In most cases it seems impossible to decide with absolute certainty which of these splits represents the division plane of the chromosomes.

Supposed, however, that a separation of the conjugated chromosomes should take place in this division, then the thorough resemblance in the genesis and the whole appearance of the chromosomes during the maturation and cleavage divisions must be considered as a mere chance. The longitudinal split of the daughter chromosomes, would have a different meaning in each of the maturation divisions and in the cleavage; in the first maturation division it would mean the split between the conjugated chromosomes, in the second it must be explained in some other way — most likely, perhaps, as a precocious splitting for the next division; in the cleavage divisions, finally, the longitudinal split is certainly not to be seen in connection with the following division as it is shown to be identical with the space between the two branches of the V-shaped chromosomes of the prophase. In the same way all the other stages in the development of the chromosomes would, in spite of their detailed resemblance, have to be explained in different ways.

Considering, on the other hand, the fact that in this species, so favorable for an examination of the chromosomes, no evidence at all is found, which might establish a proof of the existence of a reduction division — it seems to me more natural to look upon the conformity in the behavior of the chromosomes as an expres-

sion also of a corresponding series of changes going on within them during each mitosis.

Whatever, therefore, the meaning may be of the different structures of the chromosomes, they ought, I think, to be looked upon from one and the same point of view in each of the maturation divisions as in the cleavage, and the results reached in any of these divisions may be used for an explanation of similar structures in the others.

According to this view, I consider the question of a reduction division in *Nereis* as lying outside of the actual discussion until a sufficient proof for its universality is established in other species.

The questions to be settled with regard to *Nereis* concern the two other possibilities, mentioned above — whether or not the conjugating chromosomes have fused completely before the maturation divisions.

As I said before, I have not yet the material for a definite answer of this question ; and it will be the aim of my following investigations to procure such material through a comparative study of the chromosomes of the germ track in *Nereis* and those of other species.

With our present knowledge of the chromosome-relations in *Nereis* the evidences in favor of each of these views seem to balance each other.

If we take our starting point in the prophase of the first maturation division, considering the two longitudinally split halves (*a* and *a*, pp. 62–63) of the chromosomes as the conjugates being terminally attached to the spindle, then it would seem natural to look upon the quite similarly shaped chromosomes of the following divisions from the same point of view. The gradual changes in the whole appearance of the mitosis within the first 15 to 16 hours after fertilization must then be considered as the expression of a slowly proceeding fusion of the conjugating chromosomes (*a* and *a*), their tendency of a divergence during the prophases gradually decreasing, and their fusion during the anaphases becoming always more complete.

If on the other hand, we begin with the early cleavage divisions, where all the chromosomes are V- or horseshoe-shaped and with a median attachment to the fibers — then it would seem

more natural to consider the longitudinal split of the anaphases merely as an unusually narrow space between the two arms of V-shaped daughter chromosomes, and the cross-shaped chromosomes of the prophases as arisen through a precocious separation of the daughter chromosomes, connected or crossing each other on their middle point. The same view must be held also with regard to the maturation and the later cleavage divisions, the tetrads, so often met with, being considered as merely morphological structures, arisen through an approach of the two arms of a V, that is, their two longitudinally split halves, *a* and *a* must be looked upon as forming one continuous ribbon, sharply bent on its middle point. The point of attachment of the chromosomes would, from this point of view, in all divisions be a median one, the many cases in which a terminal attachment seems to be shown, being explained as artefacts.

As will be seen from the above, each of these interpretations meets with difficulties, which at the present state of our knowledge interferes with the acceptation of any of them.

In order to solve these difficulties it will be of great importance to follow the changes in the mode of attachment of the chromosomes throughout the whole germ track — and especially to compare the chromosome-relations of species in which a median attachment of the chromosomes seems to be predominant (*Tomopteris*, *Salamandra*, etc.), with those of other species, in which the chromosomes are terminally attached to the fibers.

In my following papers I shall publish my first results of such a comparative study, and even if the solution of the problem of maturation is still far away, I hope to be able to throw some new light on the question.

COLUMBIA UNIVERSITY,
March, 1907.

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TO THE PLATES, PP. 62–63.

The chromosomes represented in these plates are selected from among a great number of camera drawings which will be published in a following paper.

They are all drawn to the same scale, enlargement about 3,500 : 1.

The chromosomes of the first maturation division are continuously numbered ; some of these numbers being applied also to chromosomes of the later divisions, showing essentially the same structure as those of the first.

The letters *a* and *a* are applied to the morphologically distinguishable halves of the chromosomes, demonstrating their reappearance in each group of divisions, but without any interpretation concerning the meaning of these structures.

The relatively larger size of the chromosomes of the later cleavage divisions (7 to 15 h. aft. fertil.) may be due to their fixation in Flemming's fluid, while those of the maturation and the early cleavage are fixed in picro-acetic acid.

THE SACRUM OF THE LACERTILIA.

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The question of the morphology of the transverse processes of the sacral vertebræ of the *Lacertilia* seems never to have been definitely settled. There are extremely diverse statements in our various works on zoölogy concerning the exact nature of these processes. So far as I have been able to determine no one has ever taken up the study of the young stages of the lizards in order to determine this point. At the suggestion of Dr. S. W. Williston I have undertaken such a study while engaged in clearing the young and adult stages of a number of reptiles in the course of an extensive investigation on the epiphyses of the Reptilia.

The problem as it presents itself is whether or not the *Lacertilia* possess sacral ribs. If they do, there should be separate centers of ossification for these elements and we may confidently expect to find them in the embryonic condition. If there are no ribs, there should be no separate centers of ossification nor would sutures of separation of the ribs from the centra persist in the young of the lizards. The question as to whether the lizards ever had sacral ribs is not fully discussed. If the forerunners of the lizards had such ribs there would probably be a cartilaginous remnant of them in the embryo.

The material investigated includes the young and adult stages of representatives of five families of the *Lacertilia*, viz.: (1) Chameleonidæ — *Chameleon owenii* Grey, two specimens, one young and one adult from Batanga, German East Africa. (2) Iguanidæ — *Iguana* sp. ?, one young specimen from Mexico. *Phrynosoma douglassi hernandesi* Girard, three specimens, two young and one adult, taken in Natrona Co., Wyoming, this past summer by the writer. *Sceloporus* sp. ?, one young specimen from Mexico. *Sceloporus chrysosticus* Cope, nine specimens, eight young and one adult from Zopopan near Jalisco, Mexico on the semi-arid upland

plains collected by W. L. Tower. (3) Teiidæ — *Cnemidophorus sexlineatus* Linné, ten specimens, one adult and nine embryos from Mexico. (4) Agamidæ — *Draco volans* Linné, one adult specimen from the East Indies. (5) Helodermatidæ — *Heloderma suspectum* Cope, one adult specimen.

The specimens were cleared by the Schultze (1) method recently recommended by Dr. Mall (2) and more fully set forth by Hill (3). The method was adopted at the suggestion of Dr. Lillie and experiments have been made on clearing the young and adult stages of all groups of the Vertebrata except the fishes. The methods used are essentially those followed by Dr. Mall and need not be enumerated here. For a complete statement of the method the reader is referred especially to Hill's paper where Dr. Mall's methods are fully outlined. The Schultze method is an excellent one for demonstrating the intimate relations of the bones and cartilages of small animals and deserves to come into more general use as a method for laboratory demonstration.

Among the many recent writers on the lizards Friedrich Siebenrock seems to be the only one who has a definite conception of the true nature of the transverse processes of the sacral vertebræ of the *Lacertilia*. Cope with all of his keen insight and his wide acquaintance with fossil and recent reptiles seems not to have comprehended the unique character of the lacertilian sacrum. This seems the more remarkable since Cope did more on the lizards than any other naturalist. Cope's observations, however, are in some respects hasty and much of his work will need thorough revision. As an example of this he states on page 163 of his large work on the reptiles of North America, in speaking of the crocodiles: "There are two sacral vertebræ and no sacral ribs." But in the sacrum of the crocodiles there are no transverse processes and there *are sacral ribs*.

Siebenrock in writing on the skeleton of *Lacerta simonyi* Steind. (4) makes the following statement concerning the transverse processes of the lacertilian sacral vertebræ: "Die Frage über die morphologische Bedeutung der Querfortsätze an den Sacralwirbeln der Saurier sieht noch einer entscheidenden Lösung entgegen. Nach Gegenbaur (5) könnte man sie sowohl mit den praesacralen Rippen als auch mit den postsacralen Querfortsätze vergleichen,

so dass die Homologie zwischen Rippen und Querfortsätze ergeben wurde. Hoffman (6) glaubt jedoch annehmen zu dürfen, dass dieselben selbständig ossificiren und daher den Rippen entsprechen, obwohl der von Hoffman untersuchte *Monitor*-Embryo in der Entwickelung schon zuweit vorgeschritten war, um die Trennung der Querfortsätze vom Wirbeln constatiren zu können. Diese Trennung kann sich aber sogar an ausgewachsenen Thieren erhalten, wie von mir in drei Fallen und zwar an einem Skelete von *Hoplurus*, *Tropidurus*, und *Uromastix* wahrgenommen wurde. Denn der erste Sacralwirbel besitzt Rippen anstatt der Querfortsätze, welche dem Wirbel nicht allein durch eine Naht wie bei den Krokodilen und Schildkröten getrennt werden, sondern mit demselben sogar gelenkig verbunden sind."

In his contribution to the subject of vertebral assimilation Siebenrock (7) describes several cases such as he mentions in the above quotation and figures the condition in the sacra of *Uromastix spinipes* Merr. and *Lacerta simonyi* Steind. where the first sacral vertebra as Siebenrock calls it but which is in reality a posterior dorsal, bears a rib. Cope (8) likewise, mentions such a case as occurring in the vertebral column of *Phrynosoma* and Siebenrock has found the same condition in that genus. Such a condition, however, cannot be interpreted to mean three sacral vertebræ as Siebenrock believes. The vertebra assimilated is not a morphological sacral but merely a functional one and on that account cannot be called a true sacral. Such a condition as Siebenrock describes is of frequent occurrence among the other reptiles. The appearance of a rib in this situation is not a very remarkable occurrence since there are ribs in this situation in the primitive vertebrates and the occurrence of this rib in the lizards may confidently be regarded as a persistence of the embryonic condition in which the sacral ribs remain as vestiges. In the sacrum of *Lyriocephalus* Siebenrock (12) describes a vestige which may be interpreted to be a remnant of the sacral rib.

A close study of my material clearly shows that there are no sacral ribs in the modern lizards. The ilia are always attached directly to the *transverse processes* of the two sacral vertebræ. In the young specimen of *Chameleon owenii* Grey the transverse processes of the two sacral vertebræ are of equal size and are

very short. They arise broadly from the centra of the vertebræ. The same may be said of the adult specimen. In the *Phrynosoma* specimens a nearer approach to the ancestral condition of the vertebrate sacrum is seen. Gegenbaur (13) is of the opinion that the sacrum of the vertebrates was primitively of but one vertebra

FIG. 1. Skeleton of *Phrynosoma douglassi hernandesi*. Girard.

as is found in the modern amphibians. Such a condition is what would be expected. In *Phrynosoma* the first sacral vertebra bears large stout transverse processes with expanded ends. The second sacral bears much smaller processes but they are still larger than the processes on the succeeding pygals. These like-

wise arise from broad bases. The disparity in size between the two pairs of processes of the sacrals is such that the anterior pair appears to bear all of the burden of support (Fig. 1). In the specimen of *Sceloporus* the transverse processes are of more nearly equal size but are longer than in the *Chameleon*. In *Draco* the processes are stout and end broadly. In *Iguana* the transverse processes of the sacrals are of equal strength and have broad ends. In *Heloderma* there are broad transverse processes supporting the pelvis. In *Cnemidophorus* the sacral vertebræ bear stout transverse processes of which the anterior pair is the larger.

One would expect to find a suture of separation between the processes and the sacral centra of young lizards did such a separation really exist but even in the youngest of my specimens, *Sceloporus*, which are two days old and in which the epiphyses are just beginning to appear as minute centers of ossification, there is not the slightest indication of any separation between the sacral centra and their transverse processes. But we have more positive evidence still, that there are no sacral ribs in the lizards. In an embryo of *Cnemidophorus sexlineatus* Linné which measures 24 mm. from the tip of the snout to the base of the tail and in which the diaphyses of the limb bones are only one half ossified and the epiphyses have not appeared at all, the broad connection of the transverse processes to the centra of the vertebræ is clearly apparent. Furthermore the ossification of the processes is seen to be proceeding outward from the body of the vertebra into the transverse process so that there is no chance for a separate center of ossification in the processes. It can thus be very definitely stated that the Lacertilia occupy an isolated place among all other known reptiles in *not having any sacral ribs whatever*.

In nearly all of our modern text-books on zoölogy the statement is made that there are sacral ribs in the lizards and in none is a statement made to the contrary. Even Huxley (9) in his work on the anatomy of vertebrated animals states that there are sacral ribs but does not discuss the matter. Parker and Haswell (10) in their "Text-Book of Zoölogy" make the statement: "The sacral vertebræ have short and strong expanded processes — *the transverse processes* — which abut against the ilia,

these are separately ossified and are to be looked upon as sacral ribs." Gadow (11) in his work on "Amphibia and Reptiles" says: "The pelvis is attached to two vertebræ by means of several ribs." I have given in the bibliography, under (10), a complete list of our general works on zoölogy in which there is any statment made concerning the sacral ribs of the Lacertilia. They all agree pretty well that there are sacral ribs.

In order to be sure that there are sacral ribs among our living reptiles other than the lizards I have investigated both the young and adult stages of the turtles, crocodiles and *Sphenodon*. In a young turtle, *Chelydra*, 44 mm. in length, the separation between the vertebral centra and their sacral ribs is clearly apparent. In a young alligator, six inches in length, the sutures between the sacral ribs and the centra are clearly seen as they are also in a specimen something over four feet in length. The sutures persist in the adult of the alligator and are found in the young and adult of the *Gavialis gangeticus* Gmel., thus completely disproving Cope's statement that there are no sacral ribs in the crocodiles. In *Sphenodon* the sacral ribs are distinct. We know that among the Dinosauria the sacral ribs did not fuse with the centra until late in life. There is in the Field Museum a specimen of a young *Morosaurus*, as identified by Mr. Riggs, which is of considerable size, and yet the sacral ribs are clearly separated from the centra. Marsh has figured a similar condition in the sacrum of *Morosaurus lentus* on Plate XXXIII. of his "Dinosaurs of North America." Hatcher in his paper on *Haplocanthosaurus* (14) gives a lengthy and very interesting discussion of the sacral ribs in the Dinosauria. He expresses it as his opinion "*that there are no true sacral ribs homologous with these elements in the tailed amphibia and that the so-called ribs are really homologous with the parapophyses or inferior branches of the transverse processes.*"

But Mr. Hatcher is mistaken in his conception of the homology of these elements above mentioned. If it is true, as he states it is, that the sacral ribs (parapophyses, Hatcher) and the transverse processes of the caudal vertebræ arise from distinct ossificatory centers in the sauropod dinosaurs then we have in these animals a primitive condition and especially as regards the

caudal ribs, recalling, as it does, the condition which exists in the modern tailed amphibians (*Menopoma*, *Necturus*). McGregor (15) has described separate caudal ribs in the Phytosauria and also describes free sacral ribs for these animals. In the young specimen of *Chelydra* referred to above the caudal ribs are clearly distinct and are separated from the centra by sutures. In the Ichthyosauria (16) the ribs were free throughout the entire length of the vertebral column. Dr. Williston tells me that he has found free caudal ribs in certain plesiosaurs. In the plesiosaurs, also, Dr. Williston has recently discovered free sacral ribs. From the above enumeration it is clear that caudal ribs are not rare among the reptiles and there can be no doubt, it seems to me, that when a free structure occurs in the sacrum it can be readily homologized with both the presacral and postsacral ribs. In the primitive condition the ribs were not differentiated into dorsals, sacrals and caudals and they varied but little in size. The dorsal and sacral ribs are retained in the majority of reptiles having become functional through use or other cause while the caudal ribs which had no real function to perform have become atrophied or vestigial. Sacral ribs without doubt exist in the dinosaurs. One striking peculiarity of the sauropod dinosaur sacrum is the elongate character of the diapophyses which in many cases serve to aid the sacral ribs in the support of the ilium. This condition obtains in the sacra of *Apatosaurus* and *Brachiosaurus* at least. The presence of these diapophyses led Hatcher, without doubt, to contend that the sacral ribs were parapophyses. But so far as I can see the presence or absence of diapophyses could have no effect whatever on the character of the sacral ribs.

Paleontology helps us not at all in determining the primitive condition of the modern terrestrial lizard sacrum. Of *Paliguana* (17) from the Triassic of South Africa only a fragmentary skull is known and from this form in the Trias to *Iguanavus* (18) in the Laramie Cretaceous, a period representing a lapse of millions of years, our knowledge of the terrestrial lizards is a complete blank. Tertiary lizards are represented for the most part by very fragmentary remains and belong, according to our best authorities, to existing families or to families only recently extinct so that they offer no differences in the morphology of the sacrum from the existing forms.

Nor do the allies of the lizards, the mosasaurs, aigialosaurs and dolichosaurs, offer any clue to the primitive condition of the sacrum. In the Mosasauria, according to Dr. Williston, there is never any sacrum, but the ilia are attached directly to the transverse processes of the vertebra which is either the second or third pygal, at least in one genus (19).

The Dolichosauria differ from the Mosasauria in that the former possess a sacrum of two vertebræ (20) but so far as I have been able to determine there has never been made out in these animals any sacral ribs. In *Adriosaurus* (21) no sacral ribs can be detected because the animal is preserved on its back and no attempt has been made, so far as I am aware, to determine the presence or absence of sacral ribs in this specimen. In *Acteosaurus* (22), however, the sacrum is well exposed but shows no evidences of sacral ribs. In *Dolichosaurus* (23) Owen says: "The extremities of the sacral pleuropophyses come into contact in the *Dolichosaurus* but do not coalesce." From Owen's figure it is difficult to make out just what the condition is in the sacrum of this form. The artist has certainly drawn sacral ribs in the figure but this may have been due to fracture or to a misconception on the part of the artist. Owen makes no statement of any sacral ribs.

In the Aigialosauria from the Cretaceous of Lesina the same conditions hold in the sacrum as we have described for the other forms. Gorjanovic-Kramberger (24) figures a skeleton of *Aigialosaurus* which gives no evidence of any sacral ribs nor does the author mention any ribs as occurring in the specimen. The evidence from *Opetiosaurus* (25) is purely negative since the sacral region of the specimen was in a very poorly preserved condition.

It is an interesting question for speculation just why and how such a condition as we have described should obtain in the lizards. It seems most probable that the Lacertilia constitute a branch which came off in pre-Triassic times from some primitive diapsid stem in which the sacral ribs were functional and that later the ribs from some unknown cause became atrophied.

In conclusion I wish to express my sincere thanks to Dr. Frank R. Lillie, under whose direction this work was done, for his kindly interest in my studies and for his advice. To Mr. W.

L. Tower and Mr. R. E. Scammon I am under obligations for the material which they have very kindly placed at my disposal.

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SPERMATOGENESIS IN PHILOSAMIA CYNTHIA.

PAULINE H. DEDERER.

An investigation of spermatogenesis in *Philosamia cynthia*, a moth of the Saturnid family, was undertaken in the summer of 1905, at the suggestion of Professor Crampton, who for some years has been engaged in a statistical study of variation in this form.

Few researches have as yet been made in the spermatogenesis of Lepidoptera. Platner, in a paper published in 1886, appears to have been the first to describe the development of germ cells in this group of insects. Here, however, in his plates of *Pygæra* and *Sphinx*, he gave no details of chromosome numbers and divisions, but figured chiefly the development of cytoplasmic structures in the spermatid. Among other writers who have concerned themselves principally with the cytoplasmic aspect of development, may be mentioned Meves ('97) and La Valette ('97). Munson ('06) figures a few chromosome groups in connection with an extensive account of the development of achromatic structures in the spermatogenesis of *Papilio*. Toyama's paper on the silkworm I have not been able to obtain. The observations of Miss Stevens, published in the past year, upon the spermatocytes of the butterflies *Cacœcia* and *Euvanessa*, will be referred to later.

Material.

The life history of *Philosamia* is, briefly as follows: The eggs are laid the early part of June; develop into larvæ which pupate in September, and remain in the pupal stage until their emergence as moths the following June. The development of the spermatocytes takes place in the pupa. The testes are kidney-shaped bodies, about one eighth inch long, lying within the body cavity directly beneath the abdominal tergum. They are enveloped by voluminous yellow fat bodies, from which they can be readily distinguished by their lighter color, and compact shape. The testis (Fig. 1) is divided into four lobes, by three thin sheets of

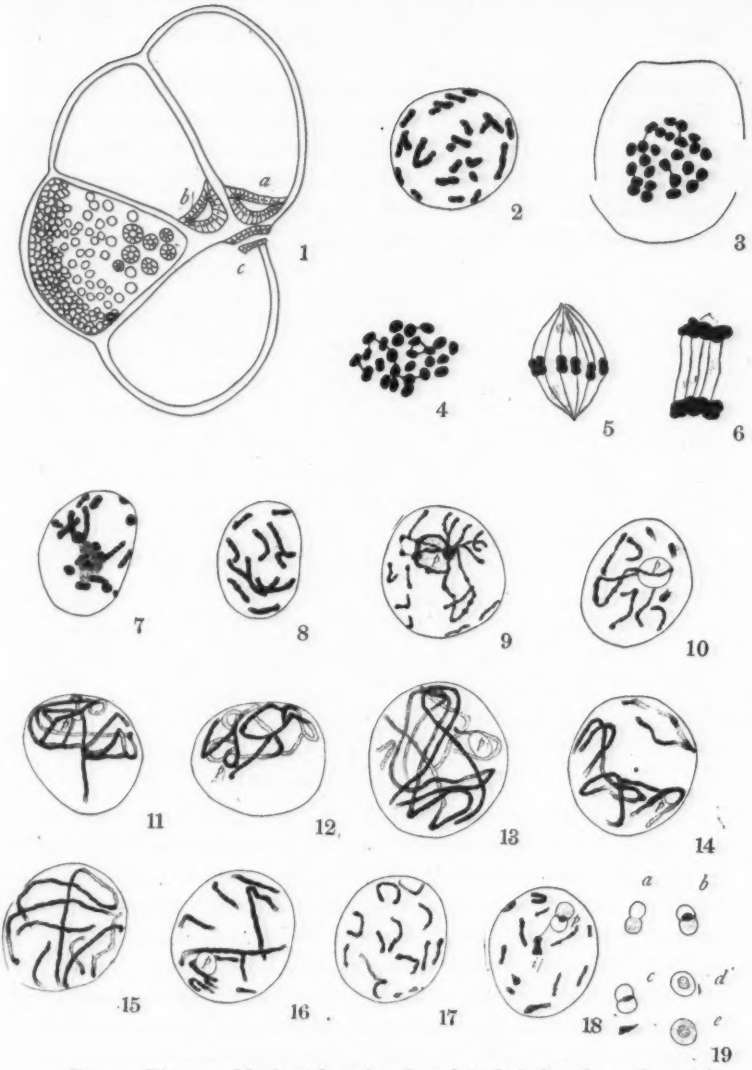


FIG. 1. Diagram of horizontal section through testis (enlarged 20 diameters) showing arrangement of cysts in the lobes. Openings of vas deferens cut obliquely at *a* and *b*, longitudinally at *c*.

FIG. 2. Early prophase of last spermatogonial division.

FIGS. 3, 4. Polar view of last spermatogonial metaphase.

FIGS. 5, 6. Side views of metaphase and of telophase (optical sections).

FIGS. 7–10. Various stages in spireme formation; appearance of plasmosome.

FIGS. 11, 12. Concentrated stage of spireme.

FIG. 13. Height of spireme stage.

FIGS. 14–16. Stages in breaking up of spireme.

FIG. 19, *a–e*. Various views of plasmosome from nuclei similar to Fig. 18.

connective tissue, which extend from the outer or convex side of the organ, and converge towards the "hilus," where each lobe opens into the sperm duct. The mature cells lie near this point. Spermatogonia are massed closely together at the opposite end, and the cells in the growth stage are grouped together in rounded cysts which lie free in the lumen of the lobe.

Methods.

Upon removal of the dorsal abdominal wall, the testes were quickly dissected out and transferred immediately to the fixing fluid. Corrosive-acetic, Gilson's alcohol-chloroform-acetic, and Flemming's fluids were used. The two former fixing agents proved especially good for spireme stages, but achromatic structures were more clearly defined with Flemming's fluid. The sections were stained with iron hæmatoxylin, with which it was possible to differentiate the plasmosome, or true nucleolus, from the chromatic nucleolus. Thionin was also used, but did not differentiate so clearly.

The figures for this paper are, with the exception of Fig. 1, from camera drawings made with compensating ocular No. 8, with a tube length of 160 mm., and $\frac{1}{12}$ oil immersion lens. They were enlarged $2\frac{1}{2}$ diameters with a drawing camera, corrected from the original, and then reduced one half in the final plates.

General Development.

Owing to the long period of development, it is not possible to find all stages of germ cells in any one testis. In material fixed during the winter, the series ranges from spermatogonia to perhaps only the first spermatocyte prophase, while in testes fixed in early June, about one week before emergence, nearly all of the cells are transformed into spermatids and spermatozoa.

There are several interesting points of general development to be observed in the winter material. A varying amount of disintegration takes place in the cells. A few first divisions appear in the autumn pupæ, but at a later period none are found, so that it is probable that these are precocious first divisions, which are followed by disintegration, while the permanent division stages appear in the spring. This observation differs from that of Wilcox, who found in *Caloptenus* that "if cells reach the spermatocyte stage they complete their course."

The cells in the growth stage also show a certain amount of disintegration, when the chromatin appears to be concentrated into one or more spherical bodies resembling yolk granules. Another point observed is that the spermatogonia along the periphery proliferate inwards a new growth at one point, forming a compact mass, the later development of which may go to make up the deficiency caused by disintegration.

In several preparations of very late stages, made in the early summer, where nearly all the cells were in the spermatid phase, small groups of giant spermatogonia were observed near the periphery of the testes. The stages ranged only from prophases to anaphases. Metaphase groups were most frequent, and several clear counts were obtained giving twenty-six chromosomes, the normal spermatogonial number. Montgomery found a similar condition in the spermatogenesis of *Peripatus*. All these giant spermatogonia were in mitosis, *i. e.*, from late prophase to anaphase. No earlier nor later stages were observed. Montgomery found that these cells were more numerous in cysts showing disintegration, and concluded that they were "hypertrophied spermatogonia, whose mitosis proceeds normally as far as the anaphase, when atrophy begins." In my preparations I have not observed that the presence of giant spermatogonia is correlated with disintegration in the cysts.

Spermatogonia.

The chromosomes of the last spermatogonial metaphase can be quite clearly seen in polar view, connected in many cases by what appear to be thin black threads (Figs. 3, 4). They are rounded bead-like bodies of approximately the same size. When turned obliquely they show a bipartite form, preparatory to the last spermatogonial division, and consequently appear larger. In the best polar views of the metaphase, twenty-six chromosomes can be seen. Only in cases where the chromatin elements are more concentrated, and hence difficult to count, does the number appear less. A side view of the equatorial plate, an optical section (Fig. 5), shows six bipartite chromosomes, arranged in a regular line at the center of the spindle. Many side views with a much larger number were seen, but this is typical of the regu-

larity of form and division of all the chromosomes. The nuclear membrane disappears at the metaphase. In telophase, the chromosomes are crowded into a dense mass, in which it is difficult to determine the outlines of individual ones (Fig. 6).

After the daughter cells have formed, and the nuclear membrane again encloses the chromosomes, the nucleus increases in

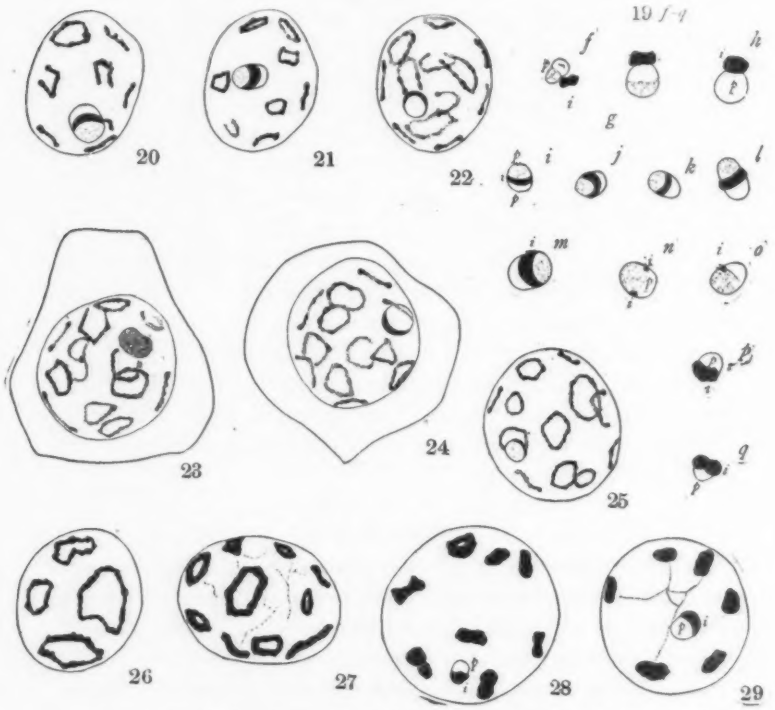


FIG. 19, *f–q*. Various views of nucleoli during growth period, showing relation of idiochromosome to plasmosome.

FIGS. 20, 21. , Early ring stage—idiochromosome attached to plasmosome.

FIGS. 22–25. Four views of later stage—showing twelve rings and nucleolus.

FIGS. 26–29. Contraction of rings into chromosomes.

size, and the chromosomes spread out into the cavity. Figs. 7, 8 show a transition from the characteristic round or oval chromosomes of the spermatogonia into rods of varying length and

uneven contour. Several of the chromosomes are grouped around a dark gray mass near the center of the nucleus, in which I believe, from what occurs later, the plasmosome appears.

The chromosomes seem to transform from rods, into longer and thinner skein-like pieces, with rougher outline, and lighter staining capacity. In Fig. 9 some of these pieces are seen to form a spireme, and this, like the chromosomes in the preceding figure, is centered around a large gray-staining mass, which is seen in Fig. 10 to be a definite plasmosome.

Spireme Stage.

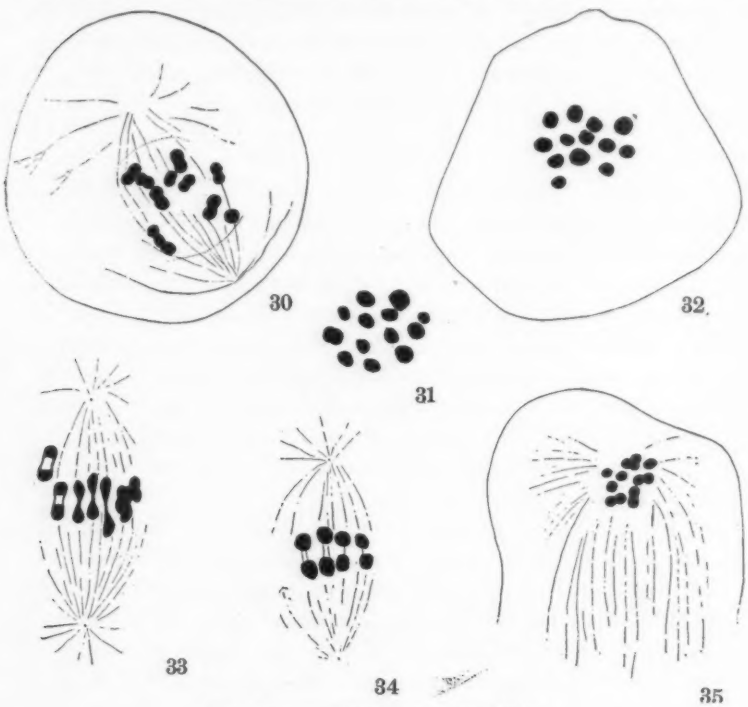
Figures 11, 12 are characteristic of the next definite stage. The chromatin pieces now form a close intricately-coiled spireme, the mass being contracted against one side of the nucleus, nearest the greatest amount of cytoplasm, as Montgomery described in *Syrbula*. The spireme is not continuous, for several free ends are seen in sections which give a complete focus of the nuclei; but it is impossible to determine the number of threads which form it. A plasmosome is seen, entangled in the meshes of the spireme. The threads are thicker and smoother in outline than in Fig. 9, and stain black even in sections which in other respects are light in color.

The question of synapsis was carefully studied in stages from 7 to 11, but I have not been able to obtain decisive evidence regarding the nature of the process. I was unable to find evidence that the chromatin rods unite definitely two by two in forming the spireme, but that they do unite in some manner seems clear. Nowhere have I found stages which might be interpreted as parasynapsis, or side by side union of the chromosomes, such as has been figured by the Schreiners, and other observers, nor could I discover that in the spireme the threads show a longitudinal split.

After contraction at one side of the nucleus, as shown in Figs. 11, 12, the spireme spreads out to occupy the entire nuclear cavity, which increases in size. Fig. 13 represents an unusually large nucleus, where the coiling of the threads, and the plasmosome, are distinctly seen.

We may speak of this condition as the height of the spireme stage. From this point onward, the spireme threads appear to

break up into shorter elements, and to become somewhat more disentangled from one another, as figured in Nos. 14 and 15. In the latter the plasmosome is not in the plane of section. In Fig. 16 this fragmentation has become more marked, until in Fig. 17, the spireme is transformed into thirteen pieces, of irregular



FIGS. 30–35. First spermatocyte division.
FIG. 30. Prophase (two chromosomes lacking in this section).
FIGS. 31, 32. Polar views of metaphase groups showing 13 chromosomes.
FIGS. 33, 34. Side views of anaphase (not all the chromosomes are shown).
FIG. 35. Telophase.

shape and outline, which are more granular and stain less intensely, than the previous stages. (The plasmosome does not appear in this section.) In Fig. 18 the limit of spireme fragmentation has been reached. Thirteen chromatin elements appear, one of which is denser than the others. The plasmosome is characteristically bipartite in this and later stages.

There is a very constant difference in the appearance of the two parts of the plasmosome. One half is clear and transparent, the other, slightly granular, and stains a deeper gray. Various views of it are shown in Fig. 19, *a* to *e*, drawn from nuclei of the same stage as Fig. 18. Three side views are given in Fig. 19, *a–c*; in the two latter the gray half seems more darkly granular at its region of union with the other. In end view, *d*, *e*, only one part is seen in outline, so that the structure appears as a single sphere, but a smaller granular area is found in the center, which seems to be nothing but the granular region indicated in *b*, seen through the clear half of the plasmosome.

The intervening history from the breaking up of the spireme into thirteen elements, and the appearance of a double plasmosome, up through the stage when rings are formed in the growth period, will be passed over for the present, to a consideration of the

Maturation Divisions.

In prophase of the first maturation division, the chromosomes appear regularly bipartite, and approximately equal in size (Fig. 30), placed irregularly upon the spindle, in preparation for metaphase. (The section lacks two of the typical number.) Many very clear metaphase groups, seen in polar view, show invariably thirteen chromosomes. Two incomplete sections (Figs. 33, 34) give views of typical anaphases, where division always appears equal.

Polar views of the second metaphase (Figs. 36, 37) have the same grouping and appearance as in the first division, the only difference being in the size of the chromosomes. In a late anaphase of second division (Fig. 38), the chromosomes of each group are approximately similar in size, and in none of the anaphases studied have I seen a case of unequal division. In early telophase the chromosomes are crowded together, and difficult to count, but several counts from polar view, or slightly oblique, showed the usual number, thirteen. Figures 39–41 are various views, all showing thirteen chromosomes as a result of second division. Fig. 42 shows the characteristic lengthening of the spindle in this division.

From the foregoing facts it is clear that in *Philosamia* there is

no odd or "accessory" chromosome, and since there seems to be also no unequal division of chromatin material, there is no element that can be distinguished as an idiochromosome-pair in the metaphase. There is, however, reason to believe that one of the bivalents differs from the others during the growth period in such a way as to indicate that it is to be identified as an equal pair of idiochromosomes, comparable with that described by Wilson in the case of *Nezara*.

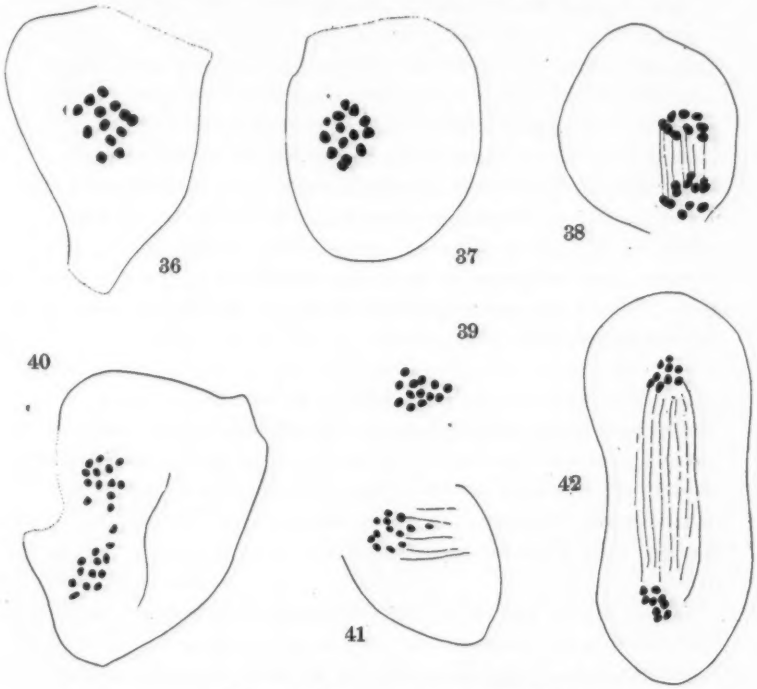
Ring Stage.

Having found twenty-six chromosomes in the spermatogonia. thirteen broken chromatin elements after the spireme stage (Figs. 17, 18), and thirteen chromosomes in maturation metaphases, I naturally expected to find that in the ring stage resulting after breaking of the spireme, thirteen rings were present. I found, however, only twelve, in a very large number of cells which I examined (Figs. 22–25). No clear case showing thirteen was observed.

My next step was to study the nucleolus present in the ring stage, to see if it might be different from the earlier double plasmosome, in containing some chromatin material. I found the double plasmosome, previously noted in Figs. 18 and 19, *a–e*, and in addition a deep black crescent-shaped band attached around the middle, its concave edge directed always toward the darker portion of the plasmosome as in Figs. 20 and 21, and 19, *m*, *n*, *o*, the two latter being views of the reverse side of the nucleolus, showing that the chromatin does not completely encircle the plasmosome. This chromatin band I interpret as equivalent to the two equal idiochromosomes found by Wilson in *Nezara*, the band thus being bivalent, as is each of the rings, and the number of chromatin elements thus forming the correct reduced number thirteen.

How, and when, does this chromatin band become associated with the plasmosome? The evidence seems to show that it is derived from one of the chromatic elements — in the case of Fig. 18, probably the darker, broader mass — and attaches itself at this stage to the plasmosome. In Fig. 19, *f*, is represented a plasmosome with the chromatin mass drawn up near it, preparatory

to attachment. The other chromatin elements in this nucleus are all irregular and feathery in outline, as in Fig. 18. Fig 19, *g*, *h*, is an end view of the same stage, showing a larger structure, with the same characteristic chromatin mass. In *i* and *j* this has become attached to the plasmosome. From the broken chromatin rods the rings are formed, apparently by a bending around of the rods; but the exact manner of the change seems very dif-



FIGS. 36–42. Second Spermatocyte Division.
FIGS. 36, 37. Polar views of metaphase groups with 13 chromosomes.
FIG. 38. Anaphase.
FIG. 39. Polar view of telophase.
FIGS. 40, 41. Late anaphase groups, side view, showing 13 chromosomes.
FIG. 42. Telophase (not all the chromosomes appear).

ficult to determine (Figs. 20, 21). In this stage, which lasts throughout the winter, the double nucleolus with its chromatin band, is a constant factor. The rings are irregular in size and

form, very granular in appearance, and stain less deeply than the spireme.

Concentration of Rings.

This is the next well-marked stage. The threads forming the rings become thicker, rougher in outline, and more deeply staining (Fig. 26), and as they thicken their circumference decreases until the originally large central space is reduced to a minute cavity, which is finally closed up altogether, and a chromosome results. Fig. 27 shows various stages in concentration. Chromosomes as shown in Fig. 28 and 29 succeed this until the at first irregular elliptical masses have assumed the smooth dumb-bell-like form seen in the first prophase stage (Fig. 30).

During concentration of the rings, the idiochromosome also appears to thicken, becoming shorter and broader, so that it has a smaller surface of contact with the plasmosome (Fig. 19, *p*, and 28). In Fig. 19, *q*, from an early prophase, it has the smooth bipartite form common to the other chromosomes. From this time, the plasmosome disappears, and the idiochromosome is indistinguishable from the others.

Conclusion.

The presence of a double idiochromosome in *Philosamia* connects it in this respect, with *Euvanessa* and *Cacœcia*, two species of butterflies studied by Miss Stevens ('06), who finds an equal pair of idiochromosomes, or "*sometimes a two lobed body*," . . . "whose only apparent peculiarity is its condensed form during growth."

In the case of the moth the idiochromosome appears single from the time of its first appearance, but it would seem that it is a *bivalent* body, in just the same way that the rings and resulting chromosomes are bivalent. This bivalence has its origin in all probability, in the prespireme stage.

Philosamia thus lies at the opposite end of a series, from *Nezara*, where the equal idiochromosomes do not unite until after the first division. An intermediate stage is represented in *Brochymena* (Wilson, '05), where the idiochromosomes, in this case unequal, lie at first separated, but later united, in the growth period. Wilson concludes for this form that, "when only one

chromatin nucleolus is present, it is to be considered as a bivalent body, arising by fusion or synapsis of the two idiochromosomes." In *Brochymena*, however, they separate again before the first division.

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Summary.

1. The spermatogonia contain twenty-six chromosomes, of approximately the same size and shape.

2. There is a definite spireme stage with a simple plasmosome.

3. The spireme segments into thirteen parts, of which twelve form rings, the thirteenth becoming attached as a chromatin mass to the plasmosome, which at this stage is double.

4. In the growth period, when the twelve rings are definitely formed, the chromatin mass is bent in a crescentic band around the plasmosome, forming a chromosome nucleolus.

5. This band represents a pair of idiochromosomes, and is bivalent like the rings, but always appears as a single body.

6. First and second metaphases show thirteen chromosomes. Divisions are equal, so that the spermatids contain similar chromosome groups.

Columbia University,
March, 1907.

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